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FIBRINOLYTIC ACTIVITY OF PASTEURELLA PESTIS

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FIBRINOLYTIC ACTIVITY OF *PASTEURELLA PESTIS*

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ABSTRACT

It has been shown previously that Pasteurella pestis produces a substance that causes dissolution of fibrin clots. Kvashina reported that this factor was found only in virulent strains and, therefore, was a necessary part of the virulence mechanism of the organism. The purpose of this study was to survey a number of strains, both virulent and avirulent, for fibrinolytic activity and to determine its relationship to virulence. A modification of the purified fibrin clot technique of Tillet and Garner was used in conjunction with the fibrin film technique of Astrup and Müllertz. Various strains of virulent and avirulent organisms were grown at 26° or 37°C under both aerobic and anaerobic conditions. Results showed that all nine virulent strains tested were capable of causing fibrin lysis both at 26° and 37°C. Contrary to the Russian findings, however, 9 of the 12 avirulent strains were also fibrinolytic. An incidental observation was also made that the fibrinolytic factor was lacking in strains that did not produce Pesticin I, a bacterocin-like substance found in most strains of P. pestis. These data do not support the finding that fibrinolytic activity is found only in virulent strains, but they indicate that it may be a factor in the organism's virulence mechanisms.

FIBRINOLYTIC ACTIVITY OF *PASTEURELLA PESTIS*

Cultivation of *Pasteurella pestis* in the peritoneal cavity of the guinea pig is a convenient method of preparing organisms that are typical of those found in natural infections.*

Virulent strains, when suspended in heart infusion broth (Difco) and inoculated at a concentration of 2×10^8 organisms are capable of killing the animal in 18 to 24 hours. The gross appearance of the animal at autopsy is shown in Figure 1. Exudate from the peritoneal cavity is homogeneous and contains large numbers of leucocytes. Although the predominating type is neutrophilic, only very few cells contain ingested organisms. One of the characteristics of this exudate is that it fails to coagulate. Figure 2 shows the result of an attempt to prepare an avirulent strain of *P. pestis* in the same manner. Note the white, fibrinous deposits around the liver. The resulting exudate shown on the right in Figure 3 was quite different in character from the virulent exudate on the left. The majority of the organisms present were engulfed by neutrophiles and coagulation occurred rapidly even in the presence of heparin; animals rarely died from this challenge.

The fact that exudates from animals infected with virulent organisms failed to coagulate, although the same type of exudate from animals injected with avirulent organisms coagulated quite rapidly, seemed to indicate that fibrinolysis might be an important factor in the virulence mechanism of *P. pestis*.

Early studies by Madison,[1] using the plasma-free fibrin clot technique of Tillet and Garner,[2] showed that all of the 16 strains of *P. pestis* tested were capable of causing destruction of various animal fibrins. No indication was given, however, of the virulence of the organisms tested.

Kvashina,[3] in a study of 20 cultures, reported that all virulent strains were fibrinolytic; avirulent strains, with the exception of EV, were not. Because this report seemed to support our original speculation, we decided to investigate the fibrinolytic properties of various virulent and avirulent strains of *P. pestis* from our stock culture collection.

Cultures were grown in a 3.7 per cent brain heart infusion (Difco) containing one per cent xylose and 0.25M sodium thiosulfate at 26°C for 24 hours with aeration by shaking. Initial tests were performed using oxalated guinea pig plasma coagulated with calcium chloride. Results were quite erratic and inconclusive; therefore, further studies were

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* In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Figure 1. Necropsy of a Guinea Pig Which had Been Injected Intraperitoneally with 10^8 Virulent P. pestis (Alexander).

Figure 2. Necropsy of a Guinea Pig Which had Been Injected Intraperitoneally with 10^8 Avirulent P. pestis (A1224).

1 2

Figure 3. Tube Number 1: Exudate From Peritoneal Cavity of Guinea Pig Injected with Virulent Organisms.
Tube Number 2: Exudate From Peritoneal Cavity of Guinea Pig Injected with Avirulent Organisms.

carried out using the purified fibrin clot technique of Tillet and Garner.[2] A typical protocol was the incubation (48 hours at 37°C) of 0.1 milliliter of test material in 0.5 milliliter (2.5 milligrams) of fibrinogen (bovine),* 0.3 milliliter of buffer (borate) pH 7.7, and 0.1 milliliter (0.1 milligram) of thrombin (bovine)** in a 10-mm by 75-mm Pyrex test tube. Various strains were selected and grown as previously described. The results of this experiment are shown in Table I. It can be seen that both virulent and avirulent strains were capable of causing fibrin lysis.

Temperature studies were conducted by growing strain A1122 at both 26° and 37°C, and testing as shown in Table II. The results indicate no apparent difference in the lytic ability of organisms grown at different temperatures; however, lysis occurred at a faster rate when the test was incubated at the higher temperature. The three negative strains plus A1122 as a positive control were grown in 250 milliliters of broth at 26°C with shaking for 24 hours. Cells were removed by centrifugation and the broth supernate was tested for lytic activity. Results indicated that the lytic factor was located chiefly in the cell itself. The cells were washed with buffer, pH 7.7, and disrupted by sonic oscillation (Raytheon, 10 k.c.) for five minutes. The debris was removed by centrifugation in the Servall SS-1 centrifuge at 32,000g for 30 minutes. Approximately a tenfold concentration resulted. The supernates were tested for fibrinolytic activity at 37°C. The results show that the negative strains still failed to cause lysis, but the activity of A1122 increased. The supernates were stored at -23°C for further study.

Figure 4 shows the result of exposing uncoagulated fibrinogen to extracts prepared in the previous experiments. Coagulation time of fibrinogen rapidly increased with increasing exposure to extract from strain A1122. After one hour, the clotting ability of fibrinogen was completely destroyed. Control clots, because of high thrombin content, lysed spontaneously after four hours. It was interesting, however, that negative control extract A12 apparently inhibited this spontaneous lysis for at least 24 hours.

The tube method of adding test materials to fibrinogen followed by coagulation with thrombin is somewhat cumbersome; therefore, the fibrin film technique of Astrup and Müllertz[4] was adopted. Films could be stored at 5°C for 48 to 72 hours before use. It was found, however, that it was necessary to expose the films to ultraviolet light for 10 to 15 minutes to prevent growth of Gram-positive, proteolytic organisms. Sensitivity of preformed fibrin appears to be equal to that of the tube test.

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* Bovine Fibrinogen, Pentex Corp.
** Bovine Thrombin, Nutritional Biochem. Corp.

TABLE I. LYSIS OF BOVINE FIBRIN BY P. PESTIS

Virulent		Avirulent	
Strain	Lysis	Strain	Lysis
ALEXANDER	+	TS	+
WASHINGTON	+	A1224	+
599	+	A1122	+
PAL	+	TJIWIDEJ	+
M23	+	SOEMDANG	+
SAKA	+	M41	+
SHASTA	+	AD5	+
YOKOHAMA	+	A4	+
POONA	+	EV76	+
		A12	-
		JAVA	-
		TRU	-

TABLE II. EFFECT OF TEMPERATURE ON THE PRODUCTION AND ACTIVITY OF THE FIBRINOLYTIC FACTOR OF P. PESTIS

Temperature of Growth	Test Incubation Temperature	Strain	Lysis Time, hours ½	1	1½	20	24	48
37°C	37°C	A1122	-	±	+	+	+	+
		A12	-	-	-	-	-	-
		TRU	-	-	-	-	-	-
		JAVA	-	-	-	-	-	-
		CONTROL	-	-	-	-	-	-
	26°C	A1122	-	-	-	+	+	+
		A12	-	-	-	-	-	-
		TRU	-	-	-	-	-	-
		JAVA	-	-	-	-	-	-
		CONTROL	-	-	-	-	-	-
26°C	37°C	A1122	-	±	+	+	+	+
		A12	-	-	-	-	-	-
		TRU	-	-	-	-	-	-
		JAVA	-	-	-	-	-	-
		CONTROL	-	-	-	-	-	-
	26°C	A1122	-	-	-	+	+	+
		A12	-	-	-	-	-	-
		TRU	-	-	-	-	-	-
		JAVA	-	-	-	-	-	-
		CONTROL	-	-	-	-	-	-

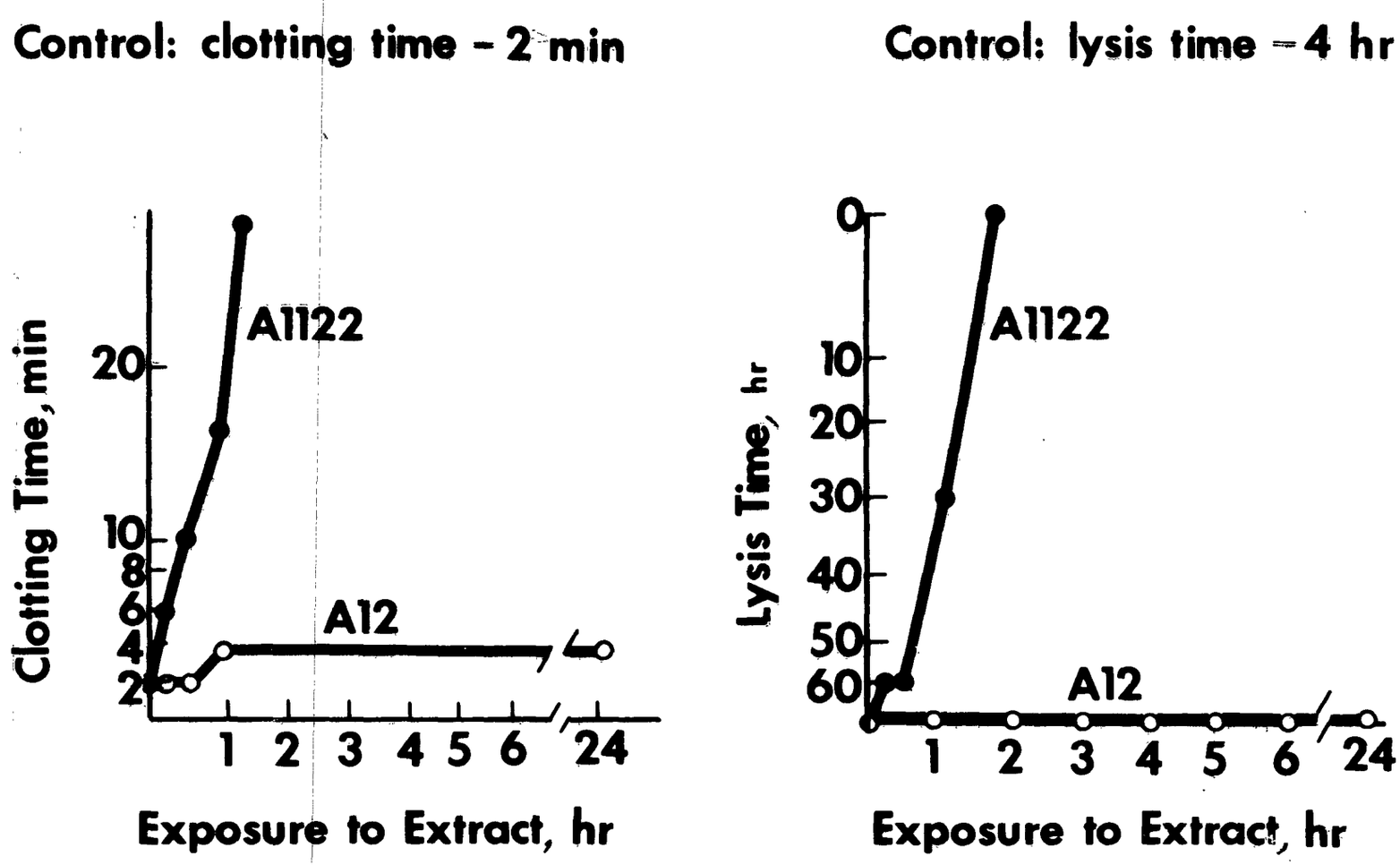

Figure 4. Fibrinogenolytic Activity of P. pestis.

This survey showed that there was a correlation between the production of the fibrinolytic factor and the occurrence of Pesticin I, reported by Brubaker and Surgalla.[5] Further studies are in progress to investigate this relationship.

A rather extensive survey of strains of P. pestis was made by G. A. Yaromyuk [Journal of Microbiol. Epidemiol. Immunobiol. (USSR) 32, 1961] and the results were similar to those presented here; of 114 strains tested, only four failed to cause lysis of fibrin. However, no methodology was presented and no indication was given concerning the virulence of the nonfibrinolytic strains.

In summary, it was found that both virulent and avirulent strains of P. pestis were capable of causing fibrin lysis. Three avirulent strains were shown to be nonfibrinolytic. These strains, also, did not produce Pesticin I. The fact that only avirulent strains have been found to lack fibrinolytic activity suggests that this process may be a necessary part of the organisms' virulence mechanism.

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